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(22) International Filing Date: 13 May 1988 (13.05.88)		(75) Inventor/Applicant (for US only) : THAISRIVONGS, Suvit [TH/US]; 1327 Edington, Portage, MI 49002 (US).	
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(71) Applicant (for all designated States except US): THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).		Published With international search report.	

**(54) Title: MALIC ACID DERIVATIVE CONTAINING RENIN INHIBITING PEPTIDES**

**(57) Abstract**

The present invention provides novel renin-inhibiting peptides of the formula  $Z-C(O)-CH(OH)-CH(CH_2R_1)-C(O)-N(R_2)-CH(CH_2R_3)-C(O)-NH-CH(CH_2R_4)-X-C(R_5)(R_2)-C(O)-Y-Z$  containing malic acid derivatives. Such inhibitors are useful for the diagnosis and control of renin-dependent hypertension and other related diseases.

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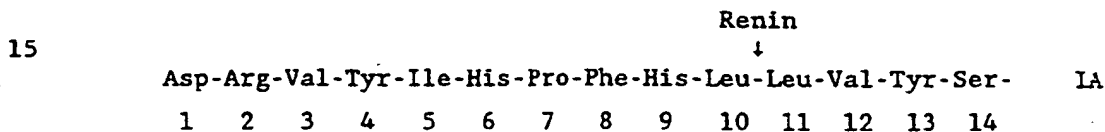
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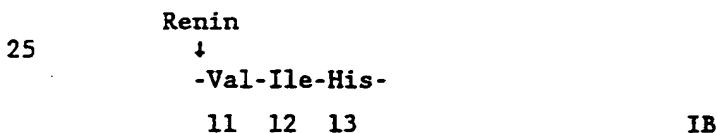
MALIC ACID DERIVATIVE-CONTAINING RENIN INHIBITING PEPTIDESDESCRIPTIONBACKGROUND OF THE INVENTION

The present invention provides novel compounds. More particularly, the present invention provides novel renin-inhibiting peptide analogs. Most particularly, the present invention provides renin-inhibitory compounds malic acid derivatives and having transition state inserts. The renin inhibitors provided herein are useful for the diagnosis and control of renin-dependent hypertension and other related diseases.

Renin is an endopeptidase which specifically cleaves a particular peptide bond of its substrate (angiotensinogen), of which the N-terminal sequence in equine substrate is for example:



as found by L. T. Skeggs et al, J. Exper. Med. 106, 439 (1957). Human renin substrate has a different sequence as recently discovered by D. A. Tewkesbury et al, Biochem. Biophys. Res. Comm. 99, 1311 (1981). It may be represented as follows:



and having the sequence to the left of the arrow (↓) being as designated in formula IA above.

Renin cleaves angiotensinogen to produce angiotensin I, which is converted to the potent pressor angiotensin II. A number of angiotensin I converting enzyme inhibitors are known to be useful in the treatment of hypertension. Inhibitors of renin are also useful in the treatment of hypertension.

A number of renin-inhibitory peptides have been disclosed. Thus, U.S. Patent 4,424,207; European published applications 45,665; 104,041; and 156,322; and U.S. patent application, Serial No. 825,250, filed 3 February 1986; disclose certain peptides with the dipeptide at the

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10,11-position containing an isosteric bond. A number of statine derivatives stated to be renin inhibitors have been disclosed, see, e.g., European published applications 77,028; 81,783; 114,993; 156,319; and 156,321; and U.S. patents 4,478,826; 4,470,971; 4,479,941; and 4,485,099. Terminal disulfide cycles have also been disclosed in renin inhibiting peptides; see, e.g., U.S. patents 4,477,440 and 4,477,441. Aromatic and aliphatic amino acid residues at the 10,11-position of the renin substrate are disclosed in U.S. patents 4,478,827 and 4,455,303. C-terminal amide cycles are disclosed in U.S. patent 4,485,099 and European published applications 156,320 and 156,318. Certain tetrapeptides are disclosed in European publications 111,266 and 77,027. Further, European published application No. 118,223 discloses certain renin inhibiting peptide analogs where the 10-11 peptide link is replaced by a one to four atom carbon or carbon-nitrogen link. Additionally, Holladay et al., in "Synthesis of Hydroxyethylene and Ketomethylene Dipeptide Isosteres", Tetrahedron Letters, Vol. 24, No. 41, pp. 4401-4404, 1983 disclose various intermediates in a process to prepare stereo-directed "ketomethylene" and "hydroxyethylene" dipeptide isosteric functional groups disclosed in the above noted U.S. Patent No. 4,424,207. Evans, et al., J. Org. Chem., 50, 4615 (1985) discloses the synthesis of Hydroxyethylene Dipeptide Isosteres. See also published European patent application 163,237 which discloses certain renin inhibiting peptides.

Additionally, published European Applications 45,161 and 53,017 disclose amide derivatives useful as inhibitors of angiotensin converting enzymes.

Certain dipeptide and tripeptides are disclosed in U.S. patents 4,514,332; 4,510,085; and 4,548,926 as well as in European published applications 128,762; 152,255; and 181,110. Pepstatin derived renin inhibitors have been disclosed in U.S. patent 4,481,192. Retroinverso bond modifications at positions 10-11 have been disclosed in U.S. patent 4,560,505 and in European published applications 127,234 and 127,235. Derivatives of isosteric bond replacements at positions 10-11 have been disclosed in European published applications 143,746 and 144,290; and U.S. patent application, Serial No. 904,149, filed 5 September 1986. Isosteric bond modifications at positions 11-12 and 12-13 have been disclosed in European published application 179,352. Certain peptides containing 2-substituted statine analogues have been

disclosed in European published application 157,409. Certain peptides containing 3-aminodeoxystatine have been disclosed in European published application, 161,588. Certain peptides containing 1-amin-2-hydroxybutane derivatives at positions 10-11 have been disclosed in  
5 European published application 172,346. Certain peptides containing 1-amino-2-hydroxypropane derivatives at positions 10-11 have been disclosed in European published application 172,347. Certain peptides containing N-terminal amide cycles have been disclosed in U.S. patent application, Serial No. 844,716, filed 27 March 1986. Certain peptides  
10 containing dihalostatine have been disclosed in PCT application, Serial No. 000,713, filed 7 April 1986. Certain peptides containing C-terminus truncated epoxy or azido or cyano groups or containing a position 10-11 diol and a position 11-12 retro bond have been disclosed in U.S. patent application, Serial No. 945,340, filed 22 December 1986.  
15 European published applications 156,322; 114,993; and 118,223; and PCT patent application, Serial No. 002,227, filed 21 November 1986; U.S. patent application, Serial No. 825,250, filed 3 February 1986; U.S. patent application, Serial No. 904,149, filed 5 September 1986; and U.S. patent application, Serial No. 844,716, filed 27 March 1986;  
20 disclose hydroxamic acids or esters at the C-terminus.

E.P. 189,203 discloses new N-dihydroxyalkyl peptide derivatives which are useful as inhibitors of renin for treating hypertension.

E.P. 184,855 discloses new hydroxy substituted-statine peptide derivatives which are useful as inhibitors of renin for treating  
25 hypertension.

Derivatives of isosteric bond replacements at positions 10-11 as dihydroxy ethylene isosteres have been disclosed in U.S. patent application, Serial No. 904,149, filed 5 September 1986.

The following references disclose additional substituents at the  
30 10, 11-position: A. Spaltenstein, P. Carpino, F. Miyake and P.B. Hyskins, Tetrahedron Letters, 27:2095 (1986); D.H. Rich and M.S. Bernatowicz, J. Med. Chem., 25:791 (1982); Roger, J. Med. Chem., 28:1062 (1985); D.M. Glick et al., Biochemistry, 21:3746 (1982); D.H. Rich, Biochemistry, 24:3165 (1985); R.L. Johnson, J. Med. Chem., 25:605  
35 (1982); R.L. Johnson and K. Verschovor, J. Med. Chem., 26:1457 (1983); R.L. Johnson, J. Med. Chem., 27:1351 (1984); P.A. Bartlett and W.B. Kezer et al., J. Am. Chem. Soc., 106:4282 (1984); Peptides: Synthesis, Structure and Function (V.J. Hruby; D.H. Rich, eds.) Proc. 8th American

Peptide Sym., Pierce Chemical Company, Rockford, Ill., pp. 511-20; 587-590 (1983).

#### INFORMATION DISCLOSURE

Certain peptides having cleavable bonds corresponding to the 10,11-position of the renin substrate and containing [malic acid derivatives are disclosed in U.S. Patent 4,629,784 (1986) Stammer; PCT Application WO 85/00809 Stammer]. Different peptides are shown to have different uses such as food additives, analgetics, CNS regulators, renin inhibitors, and antihypertensive agents.

#### SUMMARY OF THE INVENTION

The present invention provides:

The invention more particularly provides the renin inhibitory peptide of claim 2 of the Formula I

wherein X is

- (a)  $-\text{CH}(\text{OH})-$ ;
- (b)  $-\text{CH}(\text{NH}_2)-$ ;
- (c)  $-\text{C}(\text{O})-$ ;
- (d)  $-\text{CH}(\text{OH})-\text{CH}(\text{OH})-$ ;
- (e)  $-\text{CH}(\text{OH})-\text{CH}_2-$ ;
- (f)  $-\text{CH}(\text{NH}_2)-\text{CH}_2-$ ;
- (g)  $-\text{C}(\text{O})-\text{CH}_2-$ ;
- (h)  $-\text{CH}_2-\text{NH}-$ ;
- (i)  $-\text{CH}_2-\text{O}-$ ; or
- (j)  $-\text{P}(\text{O})(\text{A})\text{B}-$ ;

wherein A is

- (a)  $-\text{OH}$  or
- (b)  $-\text{NH}_2$ ;

B is

- (a) absent;
- (b)  $-\text{O}-$ ;
- (c)  $-\text{NH}-$ ; or
- (d)  $-\text{CH}_2-$ ;

wherein Y is

- (a) absent or
- (b)  $-\text{NHCH}(\text{R}_5)\text{C}(\text{O})-$ ;

wherein Z is (a)  $-\text{O}-\text{R}_6$ ; (b)  $-\text{N}(\text{R}_2)\text{R}_6$ ; or (c) Het bonded via a nitrogen atom;

wherein  $\text{R}_1$  is

- (a) hydrogen;  
(b) C<sub>1</sub>-C<sub>5</sub> alkyl;  
(c) -(CH<sub>2</sub>)<sub>p</sub>-aryl;  
(d) -(CH<sub>2</sub>)<sub>p</sub>-Het;  
5 (e) -(CH<sub>2</sub>)<sub>p</sub>-(C<sub>3</sub>-C<sub>7</sub>)cycloalkyl;  
(f) -(CH<sub>2</sub>)<sub>p</sub>-S-aryl;  
(g) -(CH<sub>2</sub>)<sub>p</sub>-S-Het;  
(h) -(CH<sub>2</sub>)<sub>p</sub>-S-(C<sub>1</sub>-C<sub>5</sub>)alkyl; or  
(i) -(CH<sub>2</sub>)<sub>p</sub>-S-(C<sub>3</sub>-C<sub>7</sub>)cycloalkyl;
- 10 wherein R<sub>2</sub> is  
(a) hydrogen; or  
(b) C<sub>1</sub>-C<sub>5</sub> alkyl;  
wherein R<sub>3</sub> is  
(a) hydrogen;  
15 (b) C<sub>1</sub>-C<sub>5</sub> alkyl;  
(c) -(CH<sub>2</sub>)<sub>p</sub>-OH;  
(d) -(CH<sub>2</sub>)<sub>p</sub>-CO<sub>2</sub>H;  
(e) -(CH<sub>2</sub>)<sub>p</sub>-NH<sub>2</sub>;  
(f) -(CH<sub>2</sub>)<sub>p</sub>-NH-C(NH<sub>2</sub>) - NH;  
20 (g) -(CH<sub>2</sub>)<sub>p</sub>-aryl;  
(h) -(CH<sub>2</sub>)<sub>p</sub>-Het; or  
(i) -(CH<sub>2</sub>)<sub>p</sub>-(C<sub>3</sub>-C<sub>7</sub>)cycloalkyl;  
wherein R<sub>4</sub> is  
(a) hydrogen;  
25 (b) C<sub>1</sub>-C<sub>5</sub> alkyl;  
(c) C<sub>3</sub>-C<sub>7</sub> cycloalkyl;  
(d) aryl;  
(e) Het;  
(f) -(CH<sub>2</sub>)<sub>p</sub>-OH; or  
30 (g) -(CH<sub>2</sub>)<sub>p</sub>-NH<sub>2</sub>;  
wherein R<sub>5</sub> is  
(a) hydrogen;  
(b) C<sub>1</sub>-C<sub>5</sub> alkyl;  
(c) -(CH<sub>2</sub>)<sub>p</sub>-aryl;  
35 (d) -(CH<sub>2</sub>)<sub>p</sub>-Het; or  
(e) -(CH<sub>2</sub>)<sub>p</sub>-(C<sub>3</sub>-C<sub>7</sub>)cycloalkyl;  
wherein R<sub>6</sub> is  
(a) hydrogen;

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- (b) aryl;  
(c) Het;  
(d) C<sub>1</sub>-C<sub>10</sub> alkyl;  
(e) -(CH<sub>2</sub>)<sub>p</sub>-(C<sub>3</sub>-C<sub>7</sub>)cycloalkyl; or  
5 (f) -(CH<sub>2</sub>)<sub>n</sub>-R<sub>7</sub>;  
wherein R<sub>7</sub> is  
(a) aryl;  
(b) Het;  
(c) hydroxy;  
10 (d) amino;  
(e) poly-hydroxylated alkyl;  
(f) -COOH;  
(g) guanidyl; or  
(h) -SO<sub>3</sub>H;  
15 wherein p is zero to 5 inclusive;  
wherein n is 1 to 5 inclusive;  
wherein aryl is phenyl or naphthyl substituted by zero to 3 of the  
following:  
(a) C<sub>1</sub>-C<sub>3</sub> alkyl;  
20 (b) hydroxy;  
(c) C<sub>1</sub>-C<sub>3</sub> alkoxy;  
(d) halogen;  
(e) amino;  
(f) mono or d-(C<sub>1</sub>-C<sub>3</sub>)alkylamino;  
25 (g) -CHO;  
(h) -CO<sub>2</sub>H;  
(i) -CO<sub>2</sub>(C<sub>1</sub>-C<sub>3</sub>)alkyl;  
(j) -CONH<sub>2</sub>;  
(k) -CONH-(C<sub>1</sub>-C<sub>3</sub>)alkyl;  
30 (l) nitro;  
(m) mercapto;  
(n) C<sub>1</sub>-C<sub>3</sub> alkythio  
(o) -SO<sub>3</sub>H;  
(p) -SO<sub>2</sub>NH<sub>2</sub>; or  
35 (q) -CN;

wherein Het is a 6 or 6-membered saturated or unsaturated ring containing from one to three heteroatoms (nitrogen, oxygen, sulfur); and including any bicyclic group in which any of the above heterocyclic



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rings is fused to a benzene ring or another heterocycle; and, if chemically feasible, the nitrogen and sulfur atoms may be in the oxidized forms; with the provisos that when X is

- (a) -CH(OH)-;
- 5 (b) -CH(NH<sub>2</sub>); and
- (c) (C(O))-;

R<sub>2</sub> and R<sub>5</sub> taken together can be -F<sub>2</sub>-; -Cl<sub>2</sub>-; -FH-; or -ClH-,

or a carboxy-, amino- or other reactive group protected form thereof;

- 10 or a pharmaceutically acceptable acid or base addition salts thereof.

By "renin inhibitory peptide" is meant a compound capable of inhibiting the renin enzyme in mammalian metabolism and linked by peptidic or pseudo-peptidic bonds.

- 15 By "a non-cleavable transition state insert" is meant a transition state insert which is not cleavable by a hydrolytic enzyme in mammalian metabolism. A variety of such transition state inserts, corresponding to the 10,11-position of the renin substrate, are known in the art, including those disclosed in the following references:

- 20 U.S. Patent 4,424,207 (Szelke); European Patent 104041A (Szelke); European Patent Application 144,290A (Ciba Geigy AG); European Patent 0,156,322 (Merck); European Patent 161-588A (Merck); European Patent 0,172,347 (Abbott); European Patent 172-346-A (Abbott); European Patent 156-318 (Merck); European Patent 157-409 (Merck); European Patent 152-255 (Sankyo); and U.S. Patent 4,548,926 (Sankyo); and

- 25 U.S. patent application, Serial No. 904,149, filed 5 September 1986; U.S. patent application, Serial No. 844,716, filed 27 March 1986; PCT application, Serial No. 000,713, filed 7 April 1986; U.S. patent application, Serial No. 945,340, filed 22 December 1986; and U.S. patent application, Serial No. 825,250, filed 3 February 1986; and

- 30 A. Spaltenstein, P. Carpino, F. Miyake and P.B. Hyskins, Tetrahedron Letters, 27:2095 (1986); D.H. Rich and M.S. Bernatowicz, J. Med. Chem., 25:791 (1982); Roger, J. Med. Chem., 28:1062 (1985); D.M. Glick et al., Biochemistry, 21:3746 (1982); D.H. Rich, Biochemistry, 24:3165 (1985); R.L. Johnson, J. Med. Chem., 25:605 (1982); R.L. Johnson and K. Verschovor, J. Med. Chem., 26:1457 (1983); R.L. Johnson, J. Med. Chem., 27:1351 (1984); P.A. Bartlett et al., J. Am. Chem. Soc., 106:4282 (1984); and Peptides: Synthesis, Structure and Function (V.J. Hruby;

D.H. Rich, eds.) Proc. 8th American Peptide Sym., Pierce Chemical Company, Rockford, Ill., pp. 511-20; 587-590 (1983).

As is apparent to those of ordinary skill in the art, the renin inhibitory peptides of the present invention can occur in several isomeric forms, depending on the configuration around the asymmetric carbon atoms. All such isomeric forms are included within the scope of the present invention. Preferably, the stereochemistry of the other amino acids corresponds to that of the naturally-occurring amino acids.

Renin inhibitory peptides commonly have protecting groups at the N-terminus and the C-terminus. These protecting groups are known in the polypeptide art. Examples of these protecting groups are given below. Any of these protecting groups are suitable for the renin inhibitory peptides of the present invention.

Furthermore, the malic acid derivative of Formula I of the present invention may occur at the N-terminus of the renin inhibitory peptide and, as such, will, when coupled with a suitable protecting group, assume the ending position.

These compounds are shown in relation to the human renin substrate as follows:

6    7    8    9    10    11    12    13  
-His Pro Phe His Leu Val Ile His-

The present invention provides peptide inhibitors of renin which are malic acid derivatives and contain at least one malic acid amino acid and have transition state inserts.

Examples of pharmaceutically acceptable acid addition salts include: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate.

The carbon atom content of various hydrocarbon-containing moieties is indicated by a prefix designating the minimum and maximum number of carbon atoms in the moiety, i.e., the prefix (C<sub>i</sub>-C<sub>j</sub>) indicates a moiety of the integer "i" to the integer "j" carbon atoms, inclusive. Thus

(C<sub>1</sub>-C<sub>4</sub>)alkyl refers to alkyl of one to 4 carbon atoms, inclusive, or methyl, ethyl, propyl, butyl, and isomeric forms thereof. C<sub>4</sub>-C<sub>7</sub> cyclic amino indicates a monocyclic group containing one nitrogen and 4 to 7 carbon atoms.

5        Examples of (C<sub>3</sub>-C<sub>10</sub>)cycloalkyl which include alkyl-substituted cycloalkyl containing a total of up to 10 total carbon atoms, are cyclopropyl, 2-methylcyclopropyl, 2,2-dimethylcyclopropyl, 2,3-diethylcyclopropyl, 2-butylcyclopropyl, cyclobutyl, 2-methylcyclobutyl, 3-propylcyclobutyl, cyclopentyl, 2,2-dimethylcyclopentyl, cyclohexyl, 10 cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and isomeric forms thereof.

      Examples of aryl include phenyl, naphthyl, (o-, m-, p-)tolyl, (o-, m-, p-)ethylphenyl, 2-ethyl-tolyl, 4-ethyl-o-tolyl, 5-ethyl-m-tolyl, (o-, m-, or p-)propylphenyl, 2-propyl-(o-, m-, or p-)tolyl, 4-isopropyl-15 2,6-xylyl, 3-propyl-4-ethylphenyl, (2,3,4- 2,3,6-, or 2,4,5-)trimethylphenyl, (o-, m-, or p-)fluorophenyl, (o-, m-, or p-)trifluoromethylphenyl, 4-fluoro-2,5-xylyl, (2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)difluorophenyl, (o-, m-, or p-)chlorophenyl, 2-chloro-p-tolyl, (3-, 4-, 5- or 6-)chloro-o-tolyl, 4-chloro-2-propylphenyl, 2-isopropyl-4-chlorophenyl, 20 4-chloro-3-fluorophenyl, (3- or 4-)chloro-2-fluorophenyl, (o-, m-, or p-)trifluoromethylphenyl, (o-, m-, or p-)ethoxyphenyl, (4- or 5-)chloro-2-methoxy-phenyl, and 2,4-dichloro(5- or 6-)methylphenyl, and the like.

      Examples of -Het include: 2-, 3-, or 4-pyridyl, imidazolyl, 25 indolyl, N<sup>in</sup>-formyl-indolyl, N<sup>in</sup>-C<sub>1</sub>-C<sub>5</sub>alkyl-C(=O)-indolyl, [1,2,4]-triazolyl, 2-, 4-, or 5-pyrimidinyl, 2- or 3-thienyl, piperidinyl, pyrrol, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, pyrazinyl, piperazinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, 30 thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, furyl, thienyl, and benzothienyl. Each of these moieties may be substituted as noted above.

      As would be generally recognized by those skilled in the art of 35 organic chemistry, a heterocycle as defined herein for -Het would not be bonded through oxygen or sulfur or through nitrogen which is within a ring and part of a double bond.

      Halo is halogen (fluoro, chloro, bromo, or iodo) or trifluoro-

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methyl.

Examples of pharmaceutically acceptable cations include: pharmacologically acceptable metal cations, ammonium, amine cations, and quaternary ammonium cations. Especially preferred metal cations are those derived from the alkali metals, e.g., lithium, sodium, and potassium, and from the alkaline earth metals, e.g., magnesium and calcium, although cationic forms of other metals, e.g., aluminum, zinc, and iron are also within the scope of this invention. Pharmacologically acceptable amine cations are those derived from primary, secondary, or tertiary amines.

The novel peptides herein contain both natural and synthetic amino acid residues. These residues are depicted using standard amino acid abbreviations (see, e.g., Eur. J. Biochem., 138, 9 (1984)) unless otherwise indicated.

In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

The renin inhibitors of this invention are useful for treating any medical condition for which it is beneficial to reduce the levels of active circulating renin. Examples of such conditions include renin-associated hypertension and hyperaldosteronism, hypertension, hypertension under treatment with another antihypertensive and/or a diuretic agent, congestive heart failure, angina, and post-myocardial infarction. The renin-angiotension system may play a role in maintenance of intracellular homeostasis: see Clinical and Experimental Hypertension, 86, 1739-1742 (1984) at page 1740 under Discussion.

Further, the renin inhibitors of this invention may be useful in the treatment of cerebrovascular disorders and disorders of intracellular homeostasis. The possible role of the renin-angiotensin system in the maintenance of intracellular homeostasis is disclosed in Clinical and Experimental Hypertension, 86:1739-1742 (1984). Additionally, the renin inhibitors of this invention potentiate the antithrombotic activity of a thromboxane antagonist (U.S. patent 4,558,037). The antihypertensive effect of the renin inhibitors of this invention are potentiated by combination with a thromboxane synthetase inhibitor.

The compounds of the present invention are preferably orally administered to humans to effect renin inhibition for the purpose of favorably affecting blood pressure. For this purpose, the compounds

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are administered from 0.1 mg to 1000 mg per kg per dose, administered from 1 to 4 times daily. The compounds of the present invention are preferably orally administered in the form of pharmacologically acceptable acid addition salts. Preferred pharmacologically acceptable salts for oral administration include the citrate and aspartate salts, although any pharmacologically acceptable salt is useful in this invention, including those listed above. These salts may be in hydrated or solvated form.

Other routes of administration include parenteral, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Equivalent dosages for such other routes of administration are thus employed. The exact dose depends on the age, weight, and condition of the patient and on the frequency and route of administration. Such variations are within the skill of the practitioner or can readily be determined.

The compounds of the present invention may be in the form of pharmaceutically acceptable salts both those which can be produced from the free bases by methods well known in the art and those with which acids have pharmacologically acceptable conjugate bases.

Conventional forms and means for administering renin-inhibiting compounds may be employed and are described, e.g., in U.S. Patent

No. 4,424,207 which is incorporated by reference herein. Likewise, the amounts disclosed in the U.S. Patent No. 4,424,207 are examples applicable to the compounds of the present invention.

5 The renin-inhibiting compounds of this invention may be administered in combination with other agents used in antihypertensive therapy such as diuretics,  $\alpha$  and/or  $\beta$ -adrenergic blocking agents, CNS-acting agents, adrenergic neuron blocking agents, vasodilators, angiotensin I converting enzyme inhibitors, and the like as described, for example, in published European patent application 156 318.

10 For example, the compounds of this invention can be given in combination with such compounds or salts or other derivative forms thereof as:

Diuretics: acetazolamide; amiloride; bendroflumethiazide; benzthiazide; bumetanide; chlorothiazide; chlorthalidone; cyclothiazide; ethacrynic acid; furosemide; hydrochlorothiazide; hydroflumethiazide; indacrinone (racemic mixture, or as either the (+) or (-) enantiomer alone, or a manipulated ratio, e.g., 9:1 of said enantiomers, respectively); metolazone; methyclothiazide; muzolimine; polythiazide; quinethazone; sodium ethacrylate; sodium nitroprusside; 20 spironolactone; ticrynaten; trimaterene; trichlormethiazide;

$\alpha$ -Adrenergic Blocking Agents: dibenamine; phentolamine; phenoxybenzamine; prazosin; tolazoline;

$\beta$ -Adrenergic Blocking Agents: atenolol; metoprolol; nadolol; propranolol; timolol;

25 (( $\pm$ )-2-[3-(tert-butylamino)-2-hydroxypropoxy]-2-furananilide) (ancarolol);

(2-acetyl-7-(2-hydroxy-3-isopropylaminopropoxy)benzofuran HCl) (befunolol);

30 (( $\pm$ )-1-(isopropylamino)-3-(p-(2-cyclopropylmethoxyethyl)-phenoxy)-2-propranol HCl) (betaxolol);

(1-[(3,4-dimethoxyphenethyl)amino]-3-(m-tolyloxy)-2-propanol HCl) (bevantalol);

(( $\pm$ )-1-(4-((2-isopropoxyethoxy)methyl)phenoxy)-3-isopropylamino-2-propanol)fumarate) (bisoprolol);

35 (4-(2-hydroxy-3-[4-(phenoxyethyl)-piperidino]-propoxy)-indole); (carbazolyl-4-oxy-5,2-(2-methoxyphenoxy)-ethylamino-2-propanol);

(1-((1,1-dimethylethyl)amino)-3-((2-methyl 'H-indol-4-yl)oxy)-2-propanol benzoate) (bopindolol);

- (1-(2-ex bicyclo[2.2.1]-hept-2-ylphenoxy)-3-[(1-methylethyl)-amino]-2-propanol HCl) (bornaprolol);
- (o-[2-hydroxy-3-[(2-indol-3-yl-1,1-dimethylethyl)-amino]propoxy]-benzonitril HCl) (bucindolol);
- 5     (α-[(tert.butylamino)methyl]-7-ethyl-2-benzofuranmethanol) (bufuralol);
- (3-[3-acetyl-4-[3-(tert.butylamino)-2-hydroxypropyl]-phenyl]-1,1-diethylurea HCl) (celiprolol);
- (±)-2-[2-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenoxy]-10 N-methylacetamide HCl) (cetamolol);
- (2-benzimidazolyl-phenyl(2-isopropylaminopropanol));
- ((±)-3'-acetyl-4'-(2-hydroxy-3-isopropylaminopropoxy)-acetanilide HCl) (diacetolol);
- (methyl-4-[2-hydroxy-3-[(1-methylethyl)aminopropoxyl]]-benzene-15 propanoate HCl) (esmolol);
- (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol);
- (1-(tert.butylamino)-3-[0-(2-propynyloxy)phenoxy]-2-propanol (pargolol);
- (1-(tert.butylamino)-3-[o-(6-hydrazino-3-pyridazinyl)phenoxy]-2-20 propanol diHCl) (prizidilol);
- ((-)-2-hydroxy-5-[(R)-1-hydroxy-2-[(R)-(1-methyl-3-phenylpropyl)-amino]ethyl]benzamide);
- (4-hydroxy-9-[2-hydroxy-3-(isopropylamino)-propoxy]-7-methyl-5H-furo[3,2-g][1]-benzopyran-5-one) (iprocrolol);
- 25     ((-)-5-(tert.butylamino)-2-hydroxypropoxy]-3,4-dihydro-1-(2H)-naphthalenone HCl) (levobunolol);
- (4-(2-hydroxy-3-isopropylamino-propoxy)-1,2-benzisothiazole HCl);
- (4-[3-(tert.butylamino)-2-hydroxypropoxy]-N-methylisocarbostyrl HCl);
- ((±)-N-2-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]ethyl-N'-30 isopropylurea) (pafenolol);
- (3-[[2-(trifluoroacetamido)ethyl]amino]-1-phenoxypropan-2-ol);
- (N-(3-(o-chlorophenoxy)-2-hydroxypropyl)-N'-(4'-chloro-2,3-dihydro-3-oxo-5-pyridazinyl)ethylenediamine);
- ((±)-N-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-35 phenyl]-butanamide) (acebutolol);
- ((±)-4'-[3-(tert.butylamino)-2-hydroxypropoxy]spiro[cyclohexane-1,2'-indan]-1'-one) (spirendolol);
- (7-[3-[[2-hydroxy-3-[(2-methylindol-4-yl)oxylpropyl]amino]-

- butyl]thio-phylline) (teopr 1ol);  
 ((±)-1-tert.butylamino-3-(thiochroman-8-yloxy)-2-propanol 1)  
 (tertato-1ol);  
 ((±)-1-tert.butylamino-3-(2,3-xylyloxy)-2-propanol HCl) (xibeno-  
 5 1ol);  
 (8-[3-(tert.butylamino)-2-hydroxypropoxy]-5-methylcoumarin)  
 (bucumo-1ol);  
 (2-(3-(tert.butylamino)-2-hydroxy-propoxy)benzonitrile HCl)  
 (bunitro-1ol);  
 10 ((±)-2'-[3-(tert-butylamino)-2-hydroxypropoxy-5'-fluorobutyro-  
 phenone) (butofilolol);  
 (1-(carbazol-4-yloxy)-3-(isopropylamino)-2-propanol) (carazolol);  
 (5-(3-tert.butylamino-2-hydroxy)propoxy-3,4-dihydrocarbotyryl HCl)  
 (carteolol);  
 15 (1-(tert.butylamino)-3-(2,5-dichlorophenoxy)-2-propanol) (clorano-  
 lol);  
 (1-(inden-4(or 7)-yloxy)-3-(isopropylamino)-2-propanol HCl)  
 (indeno-1ol);  
 (1-isopropylamino-3-[(2-methylindol-4-yl)oxy]-2-propanol) (me-  
 20 pindo-1ol);  
 (1-(4-acetoxy-2,3,5-trimethylphenoxy)-3-isopropylaminopropan-2-ol)  
 (metipranolol);  
 (1-(isopropylamino)-3-(o-methoxyphenoxy)-3-[(1-methylethyl)amino]-  
 2-propanol) (moprolol);  
 25 ((1-tert.butylamino)-3-[(5,6,7,8-tetrahydro-cis-6,7-dihydroxy-1-  
 naphthyl)oxy]-2-propanol) (nadolol);  
 ((S)-1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)amino]-2-  
 propanol sulfate (2:1)) (penbutolol);  
 (4'-[1-hydroxy-2-(amino)ethyl]methanesulfonanilide) (sotalol);  
 30 (2-methyl-3-[4-(2-hydroxy-3-tert.butylaminopropoxy)phenyl]-7-methoxy-  
 isoquinolin-1-(2H)-one);  
 (1-(4-(2-(4-fluorophenyl)ethoxy)phenoxy)-3-isopropylamino-2-  
 propanol HCl);  
 ((-)-p-[3-[(3,4-dimethoxyphenethyl)amino]-2-hydroxypropoxy]-β-  
 35 methyl-cinnamionitrile) (pacrinolol);  
 ((±)-2-(3'-tert.butylamino-2'-hydroxypropylthi)-4-(5'-carbamoyl-  
 2'-thienyl)thiazole HCl) (arotinolol);  
 ((±)-1-[p-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-(isopropyl-



- amino)-2-propanol) (cicloprolol);  
 ((±)-1-[(3-chloro-2-methylindol-4-yl)oxy]-3-[(2-phenoxyethyl)-  
 amino]-2-propanol) (indopan lol);  
 ((±)-6-[[2-[[3-(p-butoxyphenoxy)-2-hydroxypropyl]amino]ethyl]-  
 5 amino]-1,3-dimethyluracil) (pirepolol);  
 (4-(cyclohexylamino)-1-(1-naphtholenyloxy)-2-butanol);  
 (1-phenyl-3-[2-[3-(2-cyanophenoxy)-2-hydroxypropyl]aminoethyl]-  
 hydantoin HCl);  
 (3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-  
 10 benzopyran) (nipradolol);  
 Angiotensin I Converting Enzyme Inhibitors:  
 1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline (captopril);  
 (1-(4-ethoxycarbonyl-2,4(R,R)-dimethylbutanoyl)indoline-2(S)-car-  
 boxylic acid);  
 15 (2-[2-[(1-(ethoxycarbonyl)-3-phenyl-propyl]amino]-1-oxopropyl]-  
 1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid);  
 ((S)-1-[2-[(1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopro-  
 pyl]octahydro-1H-indole-2-carboxylic acid HCl);  
 (N-cyclopentyl-N-(3-(2,2-dimethyl-1-oxopropyl)thiol-2-methyl-1-  
 20 oxo-propyl)glycine) (pivalopril);  
 ((2R,4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazoli-  
 dine-carboxylic acid);  
 (1-(N-[1(S)-ethoxycarbonyl-3-phenylpropyl]-(S)-alanyl)-cis, syn-  
 octa-hydroindol-2(S)-carboxylic acid HCl);  
 25 ((-)-(S)-1-[(S)-3-mercapto-2-methyl-1-oxopropyl]indoline-2-  
 carboxylic acid);  
 ([1(S),4S]-1-[3-(benzoylthio)-2-methyl-1-oxopropyl]-4-phenylthio-  
 L-proline;  
 (3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]amino)-2,3,4,5-tetra-  
 30 hydro-2-oxo-1-(3S)-benzazepine-1-acetic acid HCl);  
 (N-(2-benzyl-3-mercaptopropanoyl)-S-ethyl-L-cysteine) and the S-  
 methyl analogue;  
 (N-(1(S)-ethoxycarbonyl-3-phenylpropyl)-L-alanyl-L-proline  
 maleate) (enalapril);  
 35 N-[1-(S)-carboxy-3-phenylpropyl]-L-alanyl-1-proline;  
 N<sup>2</sup>-[1-(S)-carboxy-3-phenylpropyl]-L-lysyl-L-proline (lysinoiril);  
 Other Antihypertensive Agents: aminophylline; cryptenamine  
 acetates and tannates; deserpidine; meremethoxylline procaine; par-

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glyline; tri-methaphan camsylate; and the like. as well as admixtures and combinations thereof.

Typically, the individual daily dosages for these combinations can range from about one-fifth of the minimally recommended clinical dosages to the maximum recommended levels for the entities when they are given singly. Coadministration is most readily accomplished by combining the active ingredients into a suitable unit dosage form containing the proper dosages of each. Other methods of coadministration are, of course, possible.

The compounds of the present invention are prepared as depicted in the charts and as described more fully in the Preparations and Examples. In the charts, Ph is used to represent the phenyl ring.

#### Chart A

Chart A describes the preparation of the acid of Formula A-5.

Diastereoselective alkylation of the known dianion of the diester of Formula A-1 with 1-bromomethylnaphthalene gives the compound of Formula A-2. Selective ester hydrolysis, followed by amide formation with morpholine, gives the ester of Formula A-4. Hydrolysis of the ester then affords the acid of Formula A-5.

#### Chart B

Chart B describes the preparation of peptides of Formulas B-3 and B-4.

The compound of Formula B-1(A-5) is coupled with the known amine (B-2) in the presence of diethylphosphoryl cyanide to give the compound of Formula B-3. The tosyl group is removed by reacting the compound of Formula B-3 with 1-hydroxybenzotriazole to yield the peptide B-4.

The malic acid derivative amino acids are incorporated into a peptide using standard coupling procedures. Should the peptide exist in a protected form, the protecting groups are removed prior to coupling. For example, a Boc group is removed from an N-terminus with trifluoroacetic acid in methylene chloride and then the malic acid derivative acid is introduced. After coupling, any remaining protecting groups are removed under standard conditions. For example a tosyl group is removed from histidine using 1-hydroxybenzotriazole in methanol.

Generally, the renin inhibiting polypeptides may be prepared by either polymer assisted or solution phase peptide synthetic procedures analogous to those described hereinafter or to those methods known in

the art. For example, the carboxylic moiety of N<sup>α</sup>-t-butyloxycarbonyl (Boc)-substituted amino acid derivatives having suitable side chain protecting groups, if necessary, may be condensed with the amino functionality of a suitably protected amino acid, peptide or polymer-bound peptide using a conventional coupling protocol such as dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) or diethylphosphoryl cyanide (DEPC) and triethylamine (Et<sub>3</sub>N) in methylene chloride or dimethylformamide. The synthetic procedures used to incorporate the novel moieties herein are analogous to those described, for example, in U.S. patents 4,424,207; 4,470,971; 4,477,440; 4,477,441; 4,478,826; 4,478,827; 4,479,941; and 4,485,099, and copending application Serial No. 753,198 filed 9 July 1985, and copending application Serial No. 825,250 filed 3 February 1986, all of which are expressly incorporated by reference herein. See, also, published European patent applications 45,161; 45,665; 53,017; 77,028; 77,029; 81,783; 104,041; 111,266; 114,993; and 118,223.

Following coupling reaction completion, the N<sup>α</sup>-Boc moiety may be selectively removed with 45% trifluoroacetic acid with or without 2% anisole (v/v) in methylene chloride. Neutralization of the resultant trifluoroacetate salt may be accomplished with 10% diisopropylethylamine or sodium bicarbonate in methylene chloride. In the case of polymer-assisted peptide synthesis, this stepwise, coupling strategy may be partially or completely automated to provide the desired peptide-polymer intermediates. Anhydrous hydrofluoric acid treatment of the peptide-polymer intermediate may then be used to effect simultaneous protecting group removal and cleavage of the peptide from its polymeric support. A notable exception to this includes N<sup>in</sup>-formyl-indolyl-substituted peptides in which the N<sup>in</sup>-formyl-indolyl moiety is stable to TFA or HF but may be removed by NH<sub>3</sub> or NaOH. Because FTrp is somewhat unstable to base in synthetic procedures, possibly causing lower yields, it may be desirable in solution phase synthesis to introduce the FTrp-containing moiety late in the synthetic sequence so that it is not exposed to such conditions.

The incorporation of N<sup>in</sup>-formyl-Trp into compounds of the present invention is easily accomplished because of the commercial availability of N<sup>α</sup>-Boc-N<sup>in</sup>-formyl-Trp-OH. However, the N<sup>in</sup>-formyl moiety may be introduced into indolyl-substituted amino acid derivatives or related compounds by reaction with HCl-formic acid as reported in the liter-

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ature, see A. Previero et al, Biochim. Biophys. Acta 147, 453 (1967); Y.C.S. Yang et al, Int. J. Peptide Protein Res. 15, 130 (1980).

Generally, methods of alkylation useful in alkylating histidine for use in the present invention are found in Cheung, S.T. et al, 5 Can. J. Chem., Vol 55, pp. 906-910 (1977). However it is now found that in Cheung, S. T. et al, methods it is critical that the reaction conditions for the alkylation of histidine be anhydrous. Further, it is now found also that during work-up instead of adding water directly to the reaction mixture, it is preferred that a buffered aqueous 10 solution be added to the reaction mixture, for example, aqueous sodium or potassium hydrogen sulfate.

Variations in the above description for starting materials, reactants, reaction conditions and required protecting groups to obtain other such N-alkylated compounds are known to an ordinarily skilled 15 chemist or are readily available in the literature.

These peptides may also be prepared by the standard solid phase techniques of Merrifield. Appropriate protecting groups, reagents, and solvents for both the solution and solid phase methods can be found in "The Peptides: Analysis, Synthesis, and Biology," Vols. 1-5, 20 eds. E. Gross and T. Meienhofer, Academic Press, NY, 1979-1983.

The compounds of the present invention may be in either free form or in protected form at one or more of the remaining (not previously protected) peptide, carboxyl, amino, hydroxy, or other reactive groups. The protecting groups may be any of those known in the 25 polypeptide art. Examples of nitrogen and oxygen protection groups are set forth in T.W. Greene, Protecting Groups in Organic Synthesis, Wiley, New York, (1981); J.F.W. McOmie, ed. Protective Groups in Organic Chemistry, Plenum Press (1973); and J. Fuhrhop and G. Benzlin, Organic Synthesis, Verlag Chemie (1983). Included among the nitrogen 30 protective groups are t-butoxycarbonyl (Boc), benzyloxycarbonyl, acetyl, allyl, phthalyl, benzyl, benzoyl, trityl and the like.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following Preparations and Examples illustrate the present invention.

35 In the Preparations and Examples below and throughout this document:

Ac is acetyl;

AMP is 2-(aminomethyl)pyridine;

- BOC is t-butoxycarbonyl;  
BOM is benzyloxymethyl;  
Bz is benzyl;  
C is centigrade;  
5 Celite is a filter aid;  
DCC is dicyclohexylcarbodiimide;  
DMF is dimethylformamide;  
EtOAc is ethyl acetate;  
g. is grams;  
10 HPLC is high performance liquid chromatography;  
I<sub>2</sub> is iodine;  
IR is infra red spectra;  
A Lindlar catalyst is a modified 5% palladium on calcium carbonate catalyst, obtained from Engelhard Industries and used for reduction;  
15 M or mol is mole;  
MBA is 2-methylbutylamino (racemic or optically active);  
MBAS is 2S-methylbutylamino;  
Me is methyl;  
min. is minute;  
20 ml is milliliter;  
MS is mass spectroscopy;  
NMHis is N $\alpha$ -methyl-L-histidine;  
NMR is nuclear magnetic resonance;  
NOAl is (1-naphthyloxy)acetyl;  
25 p-TSA salt is para-toluene sulfonic acid salt;  
Ph is phenyl;  
POA is phenoxyacetyl;  
RIP means a compound having the formula H-Pro-His-Phe-His-Phe-Phe-Val-Tyr-Lys-OH.2(CH<sub>3</sub>C(=O)OH).XH<sub>2</sub>O which is a known renin-inhibiting  
30 peptide. Skellysolve B is as defined in the Merck Index, 10th edition;  
TBDMS is t-butyldimethylsilyl;  
TFA is trifluoroacetic acid;  
THF is tetrahydrofuran;  
TLC is thin layer chromatography;  
35 Tos is p-toluenesulfonyl;  
Tr is trityl (triphenylmethyl);  
2HPA is ( $\pm$ )-(2-hydroxypropyl)amino; and  
UV is ultraviolet.

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The wedge-shape line indicates a bond which extends above the plane of the paper relative to the plane of the compound thereon.

The dotted line indicates a bond which extends below the plane of the paper relative to the plane of the compound thereon.

5 Preparation 1

Diethyl 3S-hydroxy-2R-benzylsuccinate (Formula A-2A)(Refer to Chart A)

To a stirred solution of 30.6 mmol of lithium diisopropylamide in 20 ml of dry tetrahydrofuran at -78°C under argon was slowly added a solution of 2.85 g (15 mmol) of diethyl-S-malate in 5 ml of dry tetrahydrofuran. The resulting solution was allowed to stir at -25°C for 30 minutes and then recooled to -78°C. Benzyl bromide (4.3 ml, 36.1 mmol) was added and the bath was allowed to warm slowly overnight, and then 4.6 ml of acetic acid was added. The solution was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic phase was dried (magnesium sulfate) and then concentrated. The residue was chromatographed on silica gel with 25% ethyl acetate in hexane to give 2.23 g. (8 mmol, 53%) of the title compound. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.25 (q, 6H, J = 7Hz), 3.15 (m, 3H), 4.15 (m, 5H), 7.27 (bs, 5H).

20 Preparation 2

Diethyl 3S-hydroxy-2R-(1'-naphthylmethyl)succinate (Formula A-2B)(Refer to Chart A)

According to the same procedure as in the Preparation 1, 2.85 g (15 mmol) of diethyl S-malate with 30.6 mmol of lithium diisopropylamide in tetrahydrofuran with 5 g (23 mmol) of 1-bromomethylnaphthalene afforded 3.13 g (9.5 mmol, 63%) of the title compound A. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 1.20 (t, 6H, J = 7Hz), 3.4 (m, 3H), 3.7 (m, 1H), 4.2 (m, 5H), 7.3-8.2 (m, 7H).

Preparation 3

30 1-Ethyl 3S-hydroxy-2R-benzylsuccinate (Formula A-3A)(Refer to Chart A)

To a stirred solution of 266.6 mg (0.95 mmol) of diethyl-3S-hydroxy-2R-benzylsuccinate (A-2A) in 2 ml of tetrahydrofuran was added 1 ml of a 1M aqueous potassium hydroxide solution. After one hour, the reaction mixture was partitioned between dichloromethane and 1M aqueous potassium hydrogen sulfate. The organic phase was dried (magnesium sulfate) and then concentrated to give 250 mg. of the title compound.

Preparation 4

1-Ethyl 3S-hydroxy-2R-(1'-naphthylmethyl)succinate (Formula A-3B)(Refer

to Chart A)

According to the same procedure as in the Preparation 3, 561 mg (2.0 mmol) of diethyl-3S-hydroxy-2R-(1'-naphthylmethyl)succinate (A-2B) in 4 ml of tetrahydrofuran and 2 ml of a 1M aqueous potassium hydroxide solution gave 443 mg of the title compound.

Preparation 5

Ethyl 3S-hydroxy-4-morpholino-2R-benzylsuccinate (A-4A)(Refer to Chart A)

To a stirred solution of 250 mg (0.95 mmol) of 1-ethyl-3S-hydroxy-2R-benzylsuccinate (A-3A) and 260  $\mu$ l (3.0 mmol) of morpholine in 4 ml of dichloromethane was added 175  $\mu$ l (1.1 mmol) of diethylphosphoryl cyanide. After one day, the reaction mixture was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic phase was dried (magnesium sulfate) and then concentrated. The residue was chromatographed on silica gel with 1:1 = ethyl acetate:hexane to give 130 mg (0.40 mmol, 43%) of the title compound.  $^1\text{H-NMR}(\text{CDCl}_3): \delta$  1.2 (t, 3H, J = 7 Hz), 4.15 (q, 2H, J = 7 Hz), 7.3 (bs, 5H).

Preparation 6

Ethyl 3S-hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinate (A-4B)(Refer to Chart A)

According to the same procedure as in the Preparation 3, 340 mg. (1.12 mmol) of 1-ethyl-3S-hydroxy-2R-(1'-naphthylmethyl)succinate (A-3b), 130  $\mu$ l (1.5 mmol) of morpholine, 210 ml (1.2 mmol) of diisopropylethylamine and 210  $\mu$ l (1.4 mmol) of diethylphosphoryl cyanide in 5 ml of dichloromethane afforded 310 mg (0.83 mmol, 75%) of compound A-4b.  $^1\text{H-NMR}(\text{CDCl}_3): \delta$  1.22 (t, 3H, J = 7 Hz), 4.15 (q, 2H, J = 7 Hz) 7.4-8.1 (m, 7H).

Preparation 7

3S-Hydroxy-4-morpholine-2R-benzylsuccinic acid (A-5A)(Refer to Chart A)

To a stirred solution of 130 mg (0.4 mmol) of ethyl-3S-hydroxy-4-morpholino-2R-benzylsuccinate (A-4A) in 1 ml of tetrahydrofuran was added 0.5 ml of a 1M aqueous potassium hydroxide solution. After four hours the reaction mixture was acidified with 1M aqueous potassium hydrogen sulfate and then lyophilized. The resulting residue was triturated with dichloromethane to give the title compound (5a).

Preparation 8

3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinic acid (A-5B)(Refer to Chart A)

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According to the same procedure as in Preparation 7, 310 mg (0.83 mmol) of ethyl-3S-hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinate (A-4b) in 2 ml of tetrahydrofuran and 0.91 ml of 1 M aqueous potassium hydroxide gave 280 mg of the title compound after extraction of the acidified reaction mixture with 1M aqueous potassium hydrogen sulfate.

#### Preparation 9

3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl-N<sup>im</sup>-tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridyl-methylamine (B-3A) (Refer to Chart B)

To a stirred solution of 18.7 mg (64  $\mu$ mol) of 3S-hydroxy-4-morpholine-2R-benzylsuccinate acid (A-5a) and 46.3 mg (64  $\mu$ mol) of N<sup>im</sup>-tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine in 0.6 ml of dichloromethane was added 12  $\mu$ l (69  $\mu$ mol) of diisopropylethylamine and 11  $\mu$ l (72  $\mu$ mol) of diethylphosphoryl cyanide. After 6 hours, the reaction mixture was chromatographed on silica gel with 5%-8% methanol in dichloromethane gave 25.1 mg (25  $\mu$ mol, 39%) of the title peptide (B-3a). <sup>1</sup>H-NMR was consistent with the structure.

#### Preparation 10

3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinoyl-N<sup>im</sup>-tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine (B-3B) (Refer to Chart B)

According to the same procedure as in the preparation of compound B-3a, 52.3 mg (0.15 mmol) of the 3S-hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinic acid (A-5b), 108 mg (0.15 mmol) of the peptidic amine B-2, 29  $\mu$ l (0.17 mmol) of diisopropylethylamine and 28  $\mu$ l (0.18 mmol) of diethylphosphoryl cyanide in 1 ml of dichloromethane afforded 61.9 mg (59  $\mu$ mol, 40%) of the title peptide. <sup>1</sup>H-NMR was consistent with the structure.

#### Example 1

3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethyl-amine (B-4A) (Refer to Chart B)

A solution of 25.1 mg (25  $\mu$ mol) of the 3S-hydroxy-4-morpholino-2R-benzylsuccinoyl-N<sup>im</sup>-tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridyl-methylamine (B-3a) and 14 mg (0.1 mmol) of 1-hydroxybenzotriazole in a few drops of methanol was allowed to stand at room temperature overnight. The reaction mixture



was chromatographed on silica gel with 4%–10% methanol (saturated with ammonia) in dichloromethane to give 11.9 mg (14  $\mu$ mol, 56%) of the peptide B-4A.  $^1\text{H-NMR}$  was consistent with the structure. FAB-MS:  $[\text{M} + \text{H}]^+$  at  $m/z$  = 847.5082 (calculated), 847.5089 (found); HPLC =  $k'$  = 7.7.

5 Example 2

3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine (B-4B) (Refer to Chart B)

According to the same procedure as in Example 1, 61 mg (58  $\mu$ mol) of the peptide 3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinoyl- $\text{N}^{\text{im}}$ -tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine (B-3B) and 31 mg (0.23 mmol) of 1-hydroxybenzotriazole in methanol afforded 32 mg (36  $\mu$ mol, 62%) of the peptide B-4B.  $^1\text{H-NMR}$  was consistent with the structure. FAB-MS:  $[\text{M} + \text{H}]^+$  at  $m/z$  = 897.5238 (calculated), 897.5241 (found); HPLC:  $k'$  = 8.5.

Utilizing procedures similar to those used in Examples 1 and 2 but substituting the appropriate tosylated peptide as a starting material, the following peptides are obtained:

3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-L-isoleucyl-2-pyridylmethylamine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-benzylsuccinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-benzylsuccinoyl-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-L-isoleucyl-2-pyridylmethylamine,

3S-Hydroxy-4S-morpholino-2R-benzylsuccinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-2S-methylbutylamine,

3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-2S-methylbutylamine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-benzylsuccinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methylactanoyl-2S-methylbutylamine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-benzylsuccinoyl-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-2S-methylbutylamine,

3S-Hydroxy-4-morpholino-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-L-isoleucyl-2-

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pyridylmethylanine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylanine.

5 3S-Hydroxy-4-(N-methylpiperazino)-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-L-isoleucyl-2-pyridylmethylanine,

3S-Hydroxy-4S-morpholino-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-2S-methylbutylamine,

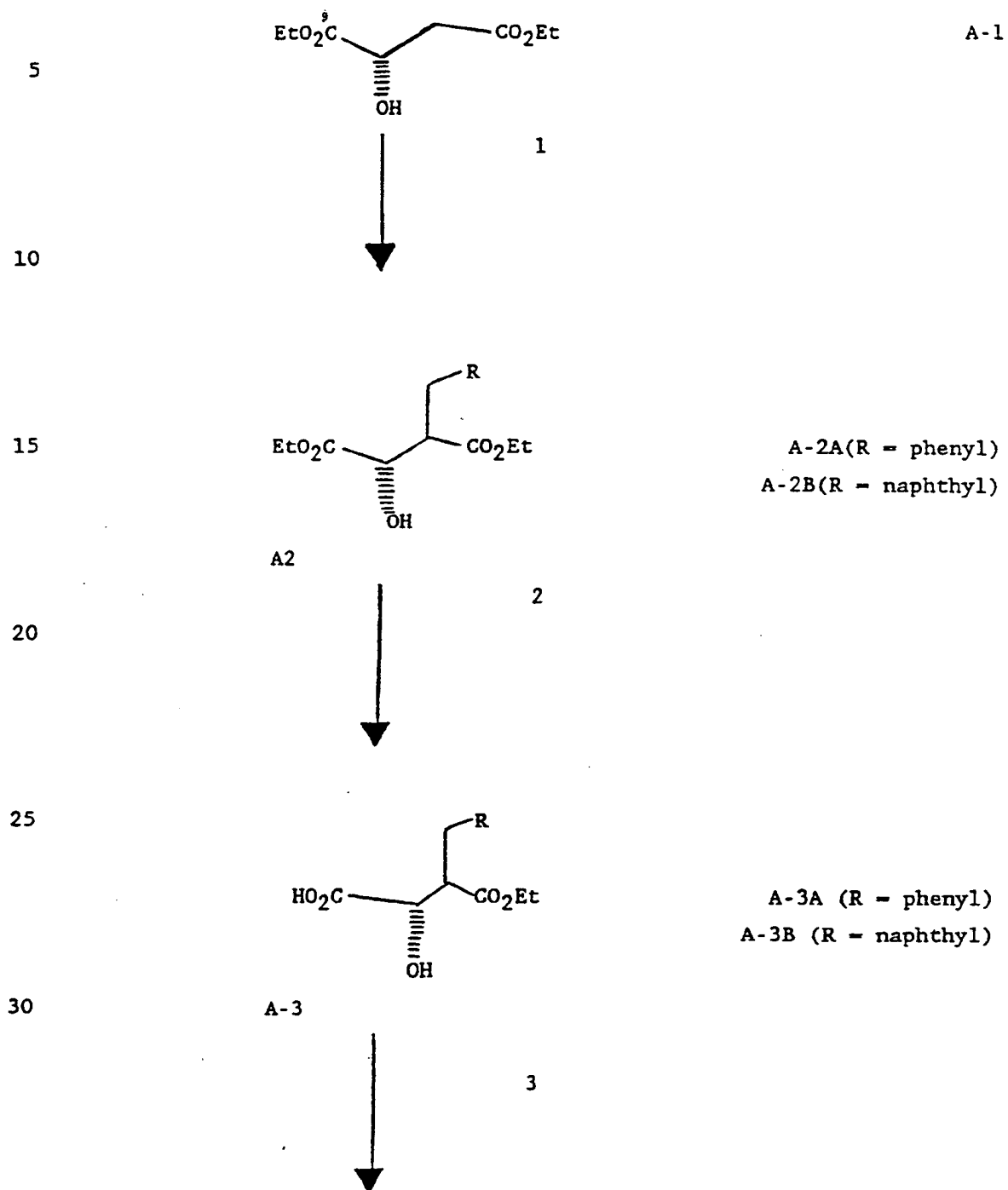
10 3S-Hydroxy-4-morpholino-2R-(1'-naphthylsuccinoyl-L-histidyl)-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-2S-methylbutylamine,

3S-Hydroxy-4-(N-methylpiperazino)-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methylactanoyl-2S-methylbutylamine,

15 3S-Hydroxy-4-(N-methylpiperazino)-2R-(1'-naphthylsuccinoyl)-L-histidyl-5S-amino-6-cyclohexyl-4S-hydroxy-2S-isopropylhexanoyl-2S-methylbutylamine.

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## CHART A



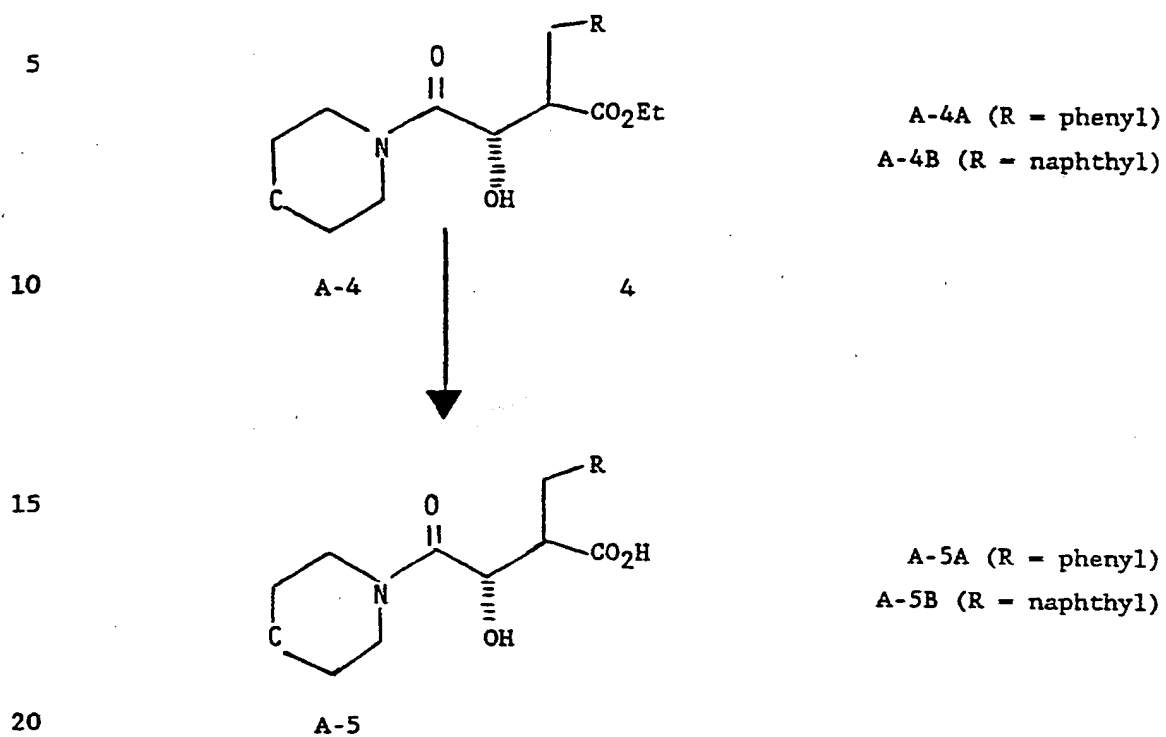
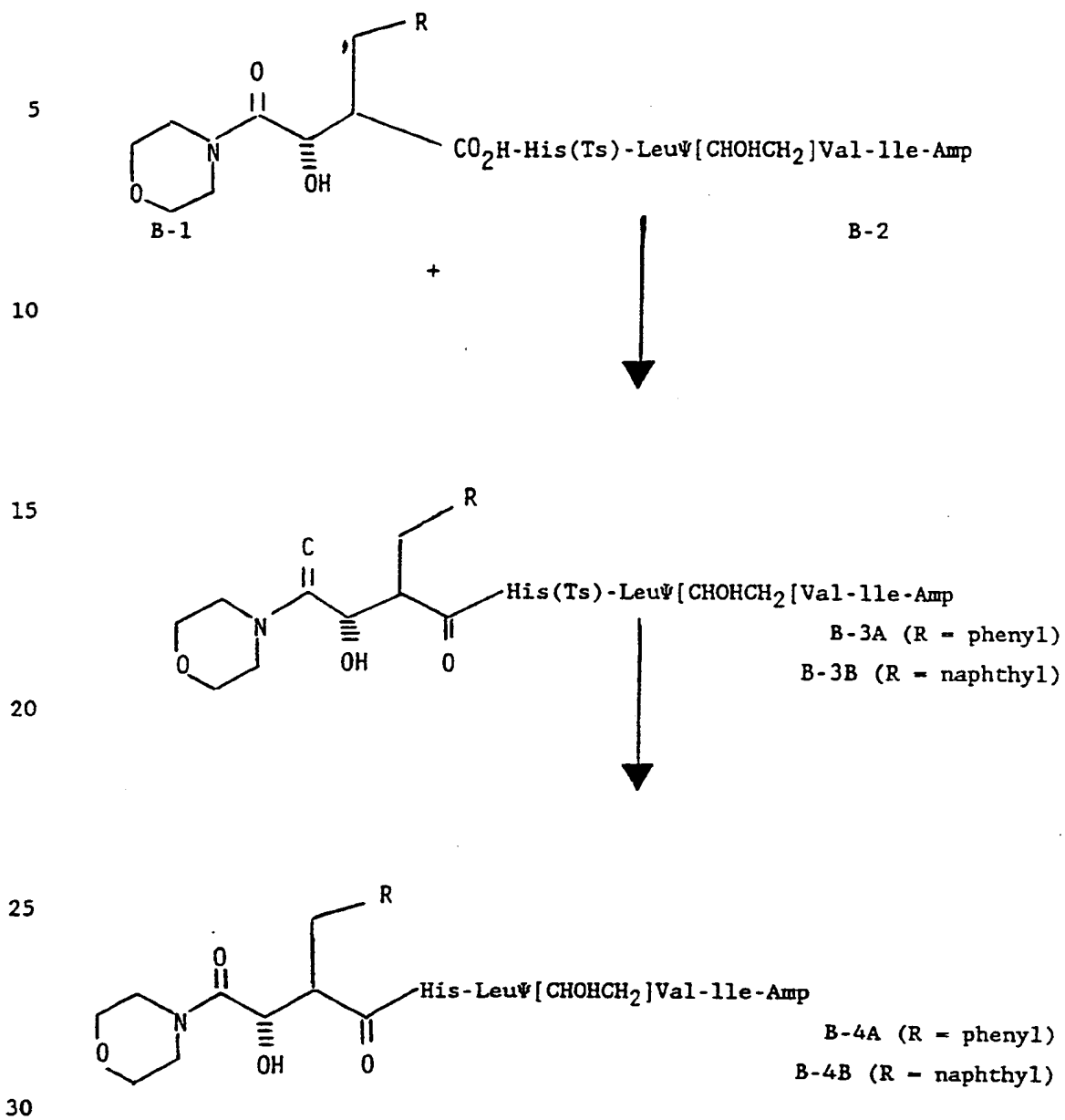


CHART B

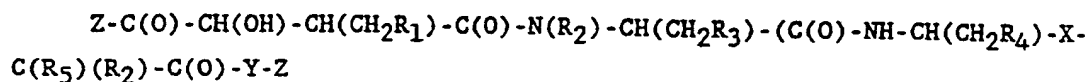


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CLAIMS

1. The renin inhibitory peptide of the Formula I

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10 wherein X is

- (a)  $-\text{CH}(\text{OH})-$ ;
- (b)  $-\text{CH}(\text{NH}_2)-$ ;
- (c)  $-\text{C}(\text{O})-$ ;
- (d)  $-\text{CH}(\text{OH})-\text{CH}(\text{OH})-$ ;
- 15 (e)  $-\text{CH}(\text{OH})-\text{CH}_2-$ ;
- (f)  $-\text{CH}(\text{NH}_2)-\text{CH}_2-$ ;
- (g)  $-\text{C}(\text{O})-\text{CH}_2-$ ;
- (h)  $-\text{CH}_2-\text{NH}-$ ;
- (i)  $-\text{CH}_2-\text{O}-$ ; or
- 20 (j)  $-\text{P}(\text{O})(\text{A})\text{B}-$ ;

wherein A is

- (a)  $-\text{OH}$  or
- (b)  $-\text{NH}_2$ ;

B is

- 25 (a) absent;
- (b)  $-\text{O}-$ ;
- (c)  $-\text{NH}-$ ; or
- (d)  $-\text{CH}_2-$ ;

wherein Y is

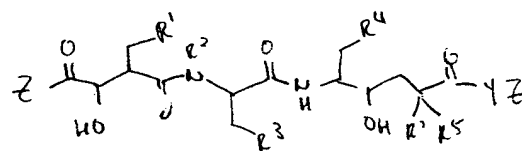
- 30 (a) absent or
- (b)  $-\text{NHCH}(\text{R}_5)\text{C}(\text{O})-$ ;

wherein Z is (a)  $-\text{O}-\text{R}_6$ ; (b)  $-\text{N}(\text{R}_2)\text{R}_6$ ; or (c) Het bonded via a nitrogen atom;

wherein  $\text{R}_1$  is

- 35 (a) hydrogen;
- (b)  $\text{C}_1-\text{C}_5$  alkyl;
- (c)  $-(\text{CH}_2)_p\text{-aryl}$ ;
- (d)  $-(\text{CH}_2)_p\text{-Het}$ ;

I



- (e)  $-(\text{CH}_2)_p-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ ;  
(f)  $-(\text{CH}_2)_p\text{-S-aryl}$ ;  
(g)  $-(\text{CH}_2)_p\text{-S-Het}$ ;  
(h)  $-(\text{CH}_2)_p\text{-S}^{\ddagger}-(\text{C}_1-\text{C}_5)\text{alkyl}$ ; or  
5 (i)  $-(\text{CH}_2)_p\text{-S}-(\text{C}_3-\text{C}_7(\text{cycloalkyl}))$ ;

wherein  $\text{R}_2$  is

- (a) hydrogen; or  
(b)  $\text{C}_1-\text{C}_5$  alkyl;

wherein  $\text{R}_3$  is

- 10 (a) hydrogen;  
(b)  $\text{C}_1-\text{C}_5$  alkyl;  
(c)  $-(\text{CH}_2)_p\text{-OH}$ ;  
(d)  $-(\text{CH}_2)_p\text{-CO}_2\text{H}$ ;  
(e)  $-(\text{CH}_2)_p\text{-NH}_2$ ;  
15 (f)  $-(\text{CH}_2)_p\text{-NH-C}(\text{NH}_2) = \text{NH}$ ;  
(g)  $-(\text{CH}_2)_p\text{-aryl}$ ;  
(h)  $-(\text{CH}_2)_p\text{-Het}$ ; or  
(i)  $-(\text{CH}_2)_p-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ ;

wherein  $\text{R}_4$  is

- 20 (a) hydrogen;  
(b)  $\text{C}_1-\text{C}_5$  alkyl;  
(c)  $\text{C}_3-\text{C}_7$  cycloalkyl;  
(d) aryl;  
(e) Het;  
25 (f)  $-(\text{CH}_2)_p\text{-OH}$ ; or  
(g)  $-(\text{CH}_2)_p\text{-NH}_2$ ;

wherein  $\text{R}_5$  is

- (a) hydrogen;  
(b)  $\text{C}_1-\text{C}_5$  alkyl;  
30 (c)  $-(\text{CH}_2)_p\text{-aryl}$ ;  
(d)  $-(\text{CH}_2)_p\text{-Het}$ ; or  
(e)  $-(\text{CH}_2)_p-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ ;

wherein  $\text{R}_6$  is

- (a) hydrogen;  
35 (b) aryl;  
(c) Het;  
(d)  $\text{C}_1-\text{C}_{10}$  alkyl;  
(e)  $-(\text{CH}_2)_p-(\text{C}_3-\text{C}_7)\text{cycloalkyl}$ ; or

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(f)  $-(CH_2)_n-R_7$ ;wherein  $R_7$  is

- (a) aryl;
- (b) Het;
- 5 (c) hydroxy;
- (d) amino;
- (e) poly-hydroxylated alkyl;
- (f)  $-COOH$ ;
- (g) guanidyl; or
- 10 (h)  $-SO_3H$ ;

wherein  $p$  is zero to 5 inclusive;wherein  $n$  is 1 to 5 inclusive;

wherein aryl is phenyl or naphthyl substituted by zero to 3 of the following:

- 15 (a)  $C_1-C_3$  alkyl;
- (b) hydroxy;
- (c)  $C_1-C_3$  alkoxy;
- (d) halogen;
- (e) amino;
- 20 (f) mono or  $d-(C_1-C_3)$ alkylamino;
- (g)  $-CHO$ ;
- (h)  $-CO_2H$ ;
- (i)  $-CO_2(C_1-C_3)$ alkyl;
- (j)  $-CONH_2$ ;
- 25 (k)  $-CONH-(C_1-C_3)$ alkyl;
- (l) nitro;
- (m) mercapto;
- (n)  $C_1-C_3$  alkythio
- (o)  $-SO_3H$ ;
- 30 (p)  $-SO_2NH_2$ ; or
- (q)  $-CN$ ;

wherein Het is a 6 or 6-membered saturated or unsaturated ring containing from one to three heteroatoms (nitrogen, oxygen, sulfur); and including any bicyclic group in which any of the above heterocyclic  
35 rings is fused to a benzene ring or another heterocycle; and, if chemically feasible, the nitrogen and sulfur atoms may be in the oxidized forms; with the provisos that when  $X$  is

- (a)  $-CH(OH)-$ ;



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(b)  $-\text{CH}(\text{NH}_2)-$ ; and(c)  $-\text{C}(\text{O})-$ ;

$\text{R}_2$  and  $\text{R}_5$  taken together can be  $-\text{F}_2-$ ;  $-\text{Cl}_2-$ ;  $-\text{FH}-$ ; or  $-\text{ClH}-$ , or a carboxy-, amino- or other reactive group protected from the  $\text{f}$ ; or a  
5 pharmaceutically acceptable acid or base addition salts thereof.

2. A compound according to claim 1, 3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl- $\text{N}^{\text{im}}$ -tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine.

10

3. A compound according to claim 1, 3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinoyl- $\text{N}^{\text{im}}$ -tosyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine.

15 4. A compound according to claim 1, 3S-Hydroxy-4-morpholino-2R-benzylsuccinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine.

20 5. A compound according to claim 2, 3S-Hydroxy-4-morpholino-2R-(1'-naphthylmethyl)succinoyl-L-histidyl-5S-amino-4S-hydroxy-2S-isopropyl-7-methyloctanoyl-L-isoleucyl-2-pyridylmethylamine.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/01535

<b>I. CLASSIFICATION F SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>4</sup> : C 07 D 401/12, // A 61 K 31/395 (C 07 D 401/12, 233:00, 213:00)												
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; text-align: left; border-bottom: 1px solid black;">Classification System</th> <th style="text-align: left; border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="vertical-align: top; border-right: 1px solid black; padding: 5px;">IPC<sup>4</sup></td> <td style="padding: 5px;">C 07 D 401/00; C 07 D 233/00; C 07 K 5/00; A 61 K 31/00; A 61 K 37/00</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div>			Classification System	Classification Symbols	IPC <sup>4</sup>	C 07 D 401/00; C 07 D 233/00; C 07 K 5/00; A 61 K 31/00; A 61 K 37/00						
Classification System	Classification Symbols											
IPC <sup>4</sup>	C 07 D 401/00; C 07 D 233/00; C 07 K 5/00; A 61 K 31/00; A 61 K 37/00											
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; text-align: left; border-bottom: 1px solid black;">Category <sup>9</sup></th> <th style="text-align: left; border-bottom: 1px solid black;">Citation of Document, <sup>10</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 10%; text-align: left; border-bottom: 1px solid black;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="vertical-align: top; border-right: 1px solid black; padding: 5px;">A</td> <td style="padding: 5px;">EP, A, 0179352 (MERCK PATENT GESELLSCHAFT) 30 April 1986 --</td> <td rowspan="3" style="vertical-align: top; border-right: 1px solid black; text-align: center; padding: 5px;">1</td> </tr> <tr> <td style="vertical-align: top; border-right: 1px solid black; padding: 5px;">A</td> <td style="padding: 5px;">WO, A, 84/03044 (FERRING AB) 16 August 1984 --</td> </tr> <tr> <td style="vertical-align: top; border-right: 1px solid black; padding: 5px;">P, A</td> <td style="padding: 5px;">EP, A, 0228192 (SANKYO) 8 July 1987, see pages 92-94; claim 1  -----</td> </tr> </table>			Category <sup>9</sup>	Citation of Document, <sup>10</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	A	EP, A, 0179352 (MERCK PATENT GESELLSCHAFT) 30 April 1986 --	1	A	WO, A, 84/03044 (FERRING AB) 16 August 1984 --	P, A	EP, A, 0228192 (SANKYO) 8 July 1987, see pages 92-94; claim 1  -----
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A	WO, A, 84/03044 (FERRING AB) 16 August 1984 --											
P, A	EP, A, 0228192 (SANKYO) 8 July 1987, see pages 92-94; claim 1  -----											
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>												
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">9th August 1988</td> <td style="border-bottom: 1px solid black; padding: 5px;">13 SEP 1988</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="padding: 5px;">EUROPEAN PATENT OFFICE</td> <td style="padding: 5px;">   <b>P.C.G. VAN DER PUTTEN</b> </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	9th August 1988	13 SEP 1988	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	 <b>P.C.G. VAN DER PUTTEN</b>		
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9th August 1988	13 SEP 1988											
International Searching Authority	Signature of Authorized Officer											
EUROPEAN PATENT OFFICE	 <b>P.C.G. VAN DER PUTTEN</b>											

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 8801535

SA 22362

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/09/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0179352	30-04-86	DE-A- 3438545	24-04-86
		AU-A- 4891785	24-04-86
		JP-A- 61100594	19-05-86
		US-A- 4709010	24-11-87
WO-A- 8403044	16-08-84	EP-A- 0118223	12-09-84
		AU-A- 2494484	30-08-84
		JP-T- 60500415	28-03-85
		AU-B- 573735	23-06-88
		US-A- 4713445	15-12-87
EP-A- 0228192	08-07-87	JP-A- 62142145	25-06-87
		JP-A- 62246546	27-10-87
		JP-A- 63063649	22-03-88

